Generation of Fluorescent Esophageal Adenocarcinoma Cells for Lineage Tracing within Composite 3D Culture Models
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ABSTRACT
While relatively rare, the aggressive nature and poor prognosis associated with esophageal carcinoma make it a particularly dangerous form of cancer. One approach to discovering esophageal cancer treatment options includes the development of composite 3D culture models. Fluorescent labeling allows each cell type within a 3D culture model to be distinguished from others, facilitating independent study via lineage tracing. Our goal was to generate fluorescent esophageal cancer cells. Of primary interest was the Homo sapiens esophageal adenocarcinoma cell line OE19 that was transfected with a plasmid carrying the mCherry (red) fluorescence marker and neomycin resistance gene. We hypothesized that if genetic antibiotic (G418) was introduced to transfected OE19 cultures, the percentage of mCherry expressing cells would be enhanced due to G418 selection against cells lacking the plasmid. To test our hypothesis, 400 µg/mL of G418 was applied to multiple OE19 passages during routine cell maintenance. Fluorescence-activated cell sorting (FACS) was then used to sort each cell based on mCherry expression. In addition, OE19 cultures under G418 selection were stained with Hoechst dye (cyan), to visualize nuclei as reference for mCherry appearance during analysis via fluorescent microscopy. The data obtained from FACS analysis indicated that 5-7% of OE19 sample cultures expressed the mCherry labeled OE19 cell line. This was supported by fluorescent microscopy. Low cell viability following cell sorting did not allow for continued culture of sorted cells. Future directions include increasing the selection pressure on OE19 cells by raising the concentration of G418 and using modified FACS procedures to improve post-sorting viability.

INTRODUCTION
Mammalian cell transfection occurs as modified, foreign DNA is introduced to a cell to generate recombinant proteins (Fig. 1). In this study, esophageal adenocarcinoma (OE19) cell cultures were transfected with a plasmid carrying the gene that encodes the fluorescent red mCherry protein and the neomycin resistance (neo) gene, which confers resistance to genetin antibiotic (G418). OE19 cells without the plasmid, and in turn mCherry and the neo gene, were terminated by G418 via treatment during routine cell passaging. By selecting for G418 resistance, surviving cell populations should carry the plasmid in abundance. In theory, these cells both resist G418 and fluoresce red, since the plasmid carries both genes. Red fluorescence of the cells allows for lineage tracing in future experiments. Fluorescent-activated cell sorting (FACS) was employed to quantify and categorize OE19 samples either positively or negatively expressive of mCherry (mCherry+ or mCherry−). To verify FACS findings, fluorescent microscopy of unsorted OE19 culture samples was employed. It is important to note that G418 concentration differs between cell lines as each vary in responsiveness to the drug, and there is a correlation between neo expression and mammalian cell growth rates in various G418 concentrations.2,3

METHODS
Fluorescence-Activated Cell Sorting (FACS)
• Prior to cell sorting, OE19 cells were resuspended in FACS buffer solution.
• Each cell was steriley and gently transported through a microfluidic cartridge to be individually analyzed and sorted on the NanoCollect Wolf Cell Sorting system (Fig. 3).

RESULTS
Fluorescent Microscopy
• Unsorted OE19 cultures were fixed with 4% paraformaldehyde and stained with Hoechst dye to visualize nuclei and assess the percentage of mCherry expressing cells.

Fig. 8. Hoechst dye nuclear staining (cyan) of transfected but unsorted OE19 cells. Cells were cultured at 400 µg/mL G418. Magnifications: 100X A, B, 200X C-C.

CONCLUSIONS
• Data from FACS analysis indicated only 5-7% mCherry expression by OE19 sample cultures.
• OE19 cells were viable post-sorting as a result of unknown causes.
• Fluorescent imaging confirmed low mCherry expression of OE19 under G418 selection.
• G418 antibiotic concentration sustained throughout the study was ineffective in enriching mCherry fluorescence in OE19 cultures

FUTURE DIRECTIONS
• Assessing effective concentrations of G418 for selective enrichment of mCherry expression in transfected OE19 cultures.
• Modifying FACS procedures to improve post-sorting cell viability for continued culturing and analysis of sorted cells.

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REFERENCES

METHODS
Cell Type of Study
• The OE19 Esophageal adenocarcinoma cell line was utilized in this study.

RESULTS
Cell Sorting
• Initial gating of the main population of cells based on forward and side scatter.

Figure 3. The process of mammalian cell transfection. The cell line of study was transfected with a plasmid encoding the mCherry protein (red fluorescence marker) and neomycin resistance gene, which confers resistance to selection with the drug G418.

Figure 2. OE19 Cell Culture. Cells are cultured in RPMI 1640 growth medium + 10% fetal bovine serum (FBS) + 1X antibiotic-antimotic (1% A/A) + 400 µg/mL G418. Image is 5 days after a 1:4 passage at 40X magnification.

Figure 4. Initial gating of the main population of cells based on forward and side scatter.

Figure 5. Gating based on forward scatter width vs. height to eliminate doublets.

Figure 6. Gating based on red fluorescence to identify and sort mCherry-expressing cells.

Figure 7. Histogram depicting the large population of mCherry-expressing cells and the small population of mCherry-expressing cells.